RPE65 in health and disease

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ZUSAMMENFASSUNG DER HABILITATIONSSCHRIFT

Zur Erlangung der Venia Legendi der Universität Zürich

Marijana Samardzija, Dr. sc. nat.

Zürich, 2015

INCLUDED ORIGINAL PUBLICATIONS

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8. Geiger, P, M Barben, C Grimm, and M Samardzija. Blue light-induced retinal lesions, intraretinal vascular leakage and edema formation in the all-cone mouse retina.
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SUMMARY

RPE65 is a protein essential for both rod- and cone-mediated vision as it regenerates the visual chromophore 11-*cis*-retinal after bleaching. In the process termed visual cycle RPE65 catalyzes a reaction in which bleached all-*trans*-retinol is isomerized to 11-*cis*-retinol. The isomerized chromophore is then taken by photoreceptor cells and recombined with the opsin moiety to restore light sensitivity of visual pigments. Without functional RPE65 trans to cis isomerization is not amenable, which in patients results in complete blindness. To date, approximately 130 pathogenic mutations have been identified in *RPE65*. Differential clinical manifestations suggest that some patients indeed suffer from null mutations leading to complete loss of vision, while others retain some useful vision due to residual RPE65 activity. The *R91W* mutation is one of the most common missense mutations found in RPE65. Patients suffering from this mutation have some useful visual function early in life, however vision is completely lost later on. To understand the mechanism of retinal degeneration and dysfunction caused by this hypomorphic RPE65 allele, we generated the *Rpe65*^{R91W} knock-in mouse (*R91W*). The impact of RPE65 insufficiency (R91W) was not only assessed in the normal, rod-dominant retina but also in a newly generated all-cone mouse (*R91W;Nrl*$^{-/-}$) thereby facilitating the understanding of rod and cone, respectively, pathophysiological mechanisms.

Publications presented here contributed to the field by significantly advancing the understanding of basic mechanisms of RPE65 physiology and it's essential role for visual pigment regeneration. Our data reinforce the notion that both rod and cone photoreceptors depend on RPE65 for chromophore supply. We provided evidence that light acting directly on diseased photoreceptor can enhance progression of retinal dystrophies. The generation and characterization of *R91W* mutant mice further improved the understanding of the pathogenic mechanisms underlying the disease caused by RPE65 insufficiency. We showed that in conditions of limited chromophore supply rods and cones compete for 11-

cis-retinal and that rods act as a sink for 11-*cis*-retinal depriving cones of the chromophre. This leads to structural destabilization of cone outer segments and a consequent cone cell death. This was further supported by a gene therapy approach where we showed that gene augmentation by the delivery of a wild type copy of the RPE65 gene led to the anatomical preservation of photoreceptors and the functional rescue of cone-mediated vision. After we have shown that the rod-dominant (*R91W*) mouse model indeed mimics many rod-related aspects of the human disease, we extended our research to the analysis of cone pathophysiology by developing the all-cone mouse (*R91W;Nrl*$^{-/-}$). The main advantage of the *R91W;Nrl*$^{-/-}$ mouse model, as opposed to most rodent models, is that the *R91W;Nrl*$^{-/-}$ outer retina is composed exclusively of cones with sustained cone visual function that can be used to study various aspects of cone degenerations. On the basis of this mouse, we generated several models (inherited and induced) to study cone degeneration in the absence of 'contaminating' rods, a situation that mimics the central macula of the human retina. All these models are highly suitable to test potential protective strategies to treat cone degenerations and macular edema.

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Publications presented here contributed to the field by significantly advancing the understanding of basic mechanisms of RPE65 physiology and it's essential role for visual pigment regeneration. Our data reinforce the notion that both rod and cone photoreceptors depend on RPE65 for chromophore supply. We provided evidence that light acting directly on diseased photoreceptor can enhance progression of retinal dystrophies. The generation and characterization of *R91W* mutant mice further improved the understanding of the pathogenic mechanisms underlying the disease caused by RPE65 insufficiency. We

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1. INTRODUCTION

Visual pigments are composed of an opsin apo-protein linked to the chromophore 11-*cis*-retinal. Visual perception begins when visual pigments of photoreceptors absorb light. Light absorption results in photoisomerization of the chromophore to all-*trans*-retinal causing a physical change in the opsin moiety and dissociation of all-*trans*-retinal from the opsin molecule. To restore light sensitivity the bleached opsin requires another molecule of 11-*cis*-retinal, which is regenerated from all-*trans*-retinal in an enzymatic pathway termed the visual cycle (Kiser et al., 2012) (**Figure1.1**). Several enzymatic steps are required for this process, but the retinal pigment epithelial protein 65 (RPE65) is the key isomerase that convers all-*trans*-retinyl ester to 11-*cis*-retinol (Jin et al., 2005, Moiseyev et al., 2005, Redmond et al., 2005). 11-*cis*-retinol dehydrogenase then oxidizes 11-*cis*-retinol to 11-*cis*-retinal (Simon et al., 1995), which is transported back to the photoreceptors and recombined with the opsin molecule.

RPE65 is located in the retinal pigment epithelium (RPE) where it constitutes up to 50% of the total microsomal proteins (Hamel et al., 1993). Although it exists as a membrane-bound and a soluble form (Xue et al., 2004), the interaction with the membrane is essential for the enzymatic activity (Nikolaeva et al., 2009). Protein sequence comparison revealed high evolutionary conservation of RPE65 with mouse and human proteins being 94% identical. The protein adopts a seven-bladed β-propeller structure as determined by x-ray crystallography (Kiser et al., 2009, Kiser et al., 2015) with an iron atom in the catalytic side coordinated by 4 conserved His residues (Poliakov et al., 2005, Redmond et al., 2005, Moiseyev et al., 2006). This nonheme ferrous iron is required for its isomerohydrolase activity (Moiseyev et al., 2006).

Reported pathogenic mutations include missense and nonsense mutations, deletions, insertions as well as mutations affecting mRNA splicing (The Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/ac/all.php). Depending on the severity of disease, the patients are diagnosed with fundus albipunctatus, early-onset retinal dystrophy, RP, or with the most severe form of inherited retinal blindness the Leber congenital amaurosis (LCA). Mutations in RPE65 account for 6 % of all LCA cases (den Hollander et al., 2008). Several animal models including the Briard dog (Aguirre et al., 1998), and mouse lines such as rd12 (Pang et al., 2005), *Rpe65*$^{-/-}$ (Redmond et al., 1998) *Rpe65 P25L* (Li et al., 2015b) and the *R91W* mouse presented here (Samardzija et al., 2008) were used to study the function of RPE65 in health and disease. These models have

been very instructive to develop treatment strategies, most importantly the AAV-mediated gene therapy approach which is currently at the Phase 1/2 clinical trial (Bainbridge et al., 2008, Cideciyan et al., 2008, Hauswirth et al., 2008, Maguire et al., 2008, Cideciyan et al., 2013, Bainbridge et al., 2015).

Here I present data describing the role of RPE65 in physiological and pathophysiological conditions and discuss the findings in relation to the literature. The data presented was acquired by studying our newly developed mouse models during the past years in the Laboratory of Retinal Cell Biology. Each of the three subsection in the results presents the most important findings related to the topic of the section and illustrates the data with a representative figure taken from one of the articles.

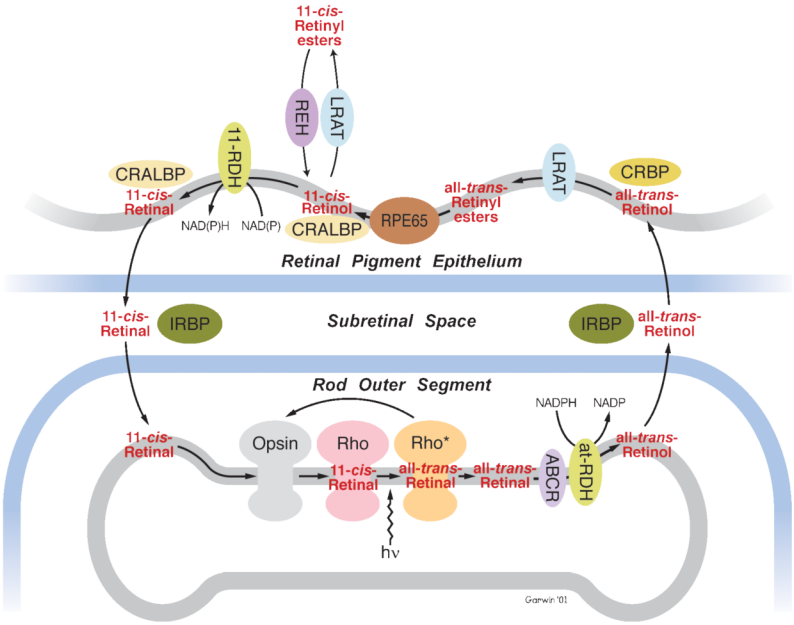

Figure 1.1. RPE65 and visual cycle

Upon photon absorption 11-*cis*-retinal is converted into all-*trans*-retinal and released into the disc lumen. The transport across the disc membrane is facilitated by ABCR and the aldehyde is reduced into all-*trans*-retinol by an atRDH. All-*trans*-retinol leaves the photoreceptor by so far unknown mechanisms and is chaperoned by IRBP in the extracellular space. It enters the RPE – again by unknown mechanisms – in the lumen of which it is chaperoned and transported by CRBP. All-*trans*-retinol is esterified to a fatty acid by LRAT. The resulting all-*trans*-retinyl ester is the storage form for vitamin A in the eye and the substrate for RPE65 that hydrolyses the all-*trans*- retinol from the ester and induces the re-isomerization to 11-*cis*-retinol. 11-*cis*-retinol binds to CRALBP and in a subsequent step is oxidized by a 11cRDH to form 11-*cis*-retinal. Alternatively, 11-*cis*-retinol may be esterified by LRAT to yield 11-*cis*-retinyl esters, a storage form of pre-isomerized retinoid. 11-*cis*-retinyl esters are hydrolyzed to 11-*cis*-retinal by 11cREH. 11-*cis*-retinal leaves the RPE, is chaperoned by IRBP during its transport back to the photoreceptor and - by unknown mechanisms – reaches the naked opsin to restore its light-sensitivity.

11cRDH, 11-*cis*-retinol dehydrogenase; 11cREH, 11-*cis*-retinyl ester hydrolase; ABCR, ATP-binding cassette retina; atRDH, all-*trans*-retinol dehydrogenase; CRALBP, cellular retinaldehyde binding protein; CRBP, cellular retinol binding protein; IRBP, inter-photoreceptor retinol binding protein; LRAT, lecithin retinol acyl transferase; RPE, retinal pigment epithelium. Adapted from (Saari, 2001)

2. RESULTS AND DISUSSION

2.1. RPE65 AS A MODIFIER GENE FOR RETINAL DEGENERATIONS

1. Wenzel, A, C Grimm, M Samardzija, and CE Reme. The genetic modifier Rpe65Leu(450): effect on light damage susceptibility in c-Fos-deficient mice.
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Life-long exposure to environmental light is implicated as cofactor important for progression of AMD as well as of inherited retinal degenerations in human patients (Margrain et al., 2004). Exposure of animal models to high levels of visible light has been used over years to study signaling pathways leading to retinal degeneration (reviewed in (Wenzel et al., 2005a)). Most importantly, excessive light induces apoptotic photoreceptor cell death followed by degeneration and loss of retinal function, as typically seen also in human retinal dystrophies. In a mouse model for Retinitis Pigmentosa (VPP) it was shown that that progression of a mutation-mediated retinal degeneration can be accelerated by light (Naash et al., 1996). This further reinforces the importance of light as an environmental factor influencing the severity and progression of blinding diseases.

The articles summarized here (Wenzel et al., 2003, Wenzel et al., 2005b, Samardzija et al., 2006) address the consequences of a sequence variant found in the *Rpe65* gene at position 450 and its influence on light damage susceptibility. Danciger et al. previously showed that this sequence variant results in an amino acid substitution at position 450 (Leu450Met) in RPE65 and leads to increased resistance against acute light damage resulting in reduced photoreceptor loss in *B6* mice (Danciger et al., 2000). We analyzed this variant in a mouse model lacking *c-fos* to investigate the significance of Danciger's finding. *c-fos* was one of the first identified genes that are crucial for light-induced retinal degeneration as its absence confers resistance against light damage (Hafezi et al., 1997).

We showed that replacing the *Met450* variant of *Rpe65* by the *Leu450* variant in *c-fos* knock out mice resulted in increased levels of RPE65 protein, accelerated rhodopsin regeneration and restored light damage susceptibility. This showed that the *Leu450* variant is sufficient to overcome the protection against light-induced damage conferred by absence of *c-fos*. Light induced retinal degeneration models served during the years as platforms to test neuroprotective compounds (Wenzel et al., 2005a). Understanding that Met450 variant leads to slower chromophore synthesis, which increases retinal resistance to light damage (Wenzel et al., 2003, Wenzel et al., 2005b) is important, as genetically modified mouse models are very commonly on a *B6* background with the light-resistant *Rpe65* variant. Therefore, such models have to be used cautiously when employed to assess potential therapeutic agents against light-induced degeneration of photoreceptors.

Most importantly we showed (Samardzija et al., 2006) that increased absorption of photons and light sensitivity, conferred by the *Leu450* variant, further accelerates retinal degeneration in the *VPP* mouse, a genetic model for Retinitis Pigmentosa (**Figure 2.1**, (Samardzija et al., 2006)). This suggested that sequence variations in the *RPE65* gene could act as genetic modifiers in inherited retinal degenerations, presumably by regulating the daily rate of photon absorption through the modulation of rhodopsin regeneration kinetics. It is interesting to note that humans are exclusively carrying *Leu450* variant and that no pathogenic mutation was identified on that amino acid residue. A recently reported mouse model carrying a human P25L missense mutation in *Rpe65* (Li et al., 2015b) shows also resistance to light-induced retinal damage due to the reduced expression and catalytic activity of the mutant RPE65. This is consistent to our notion of the importance of RPE65 as a potential modulator responsible for variations in disease progression.

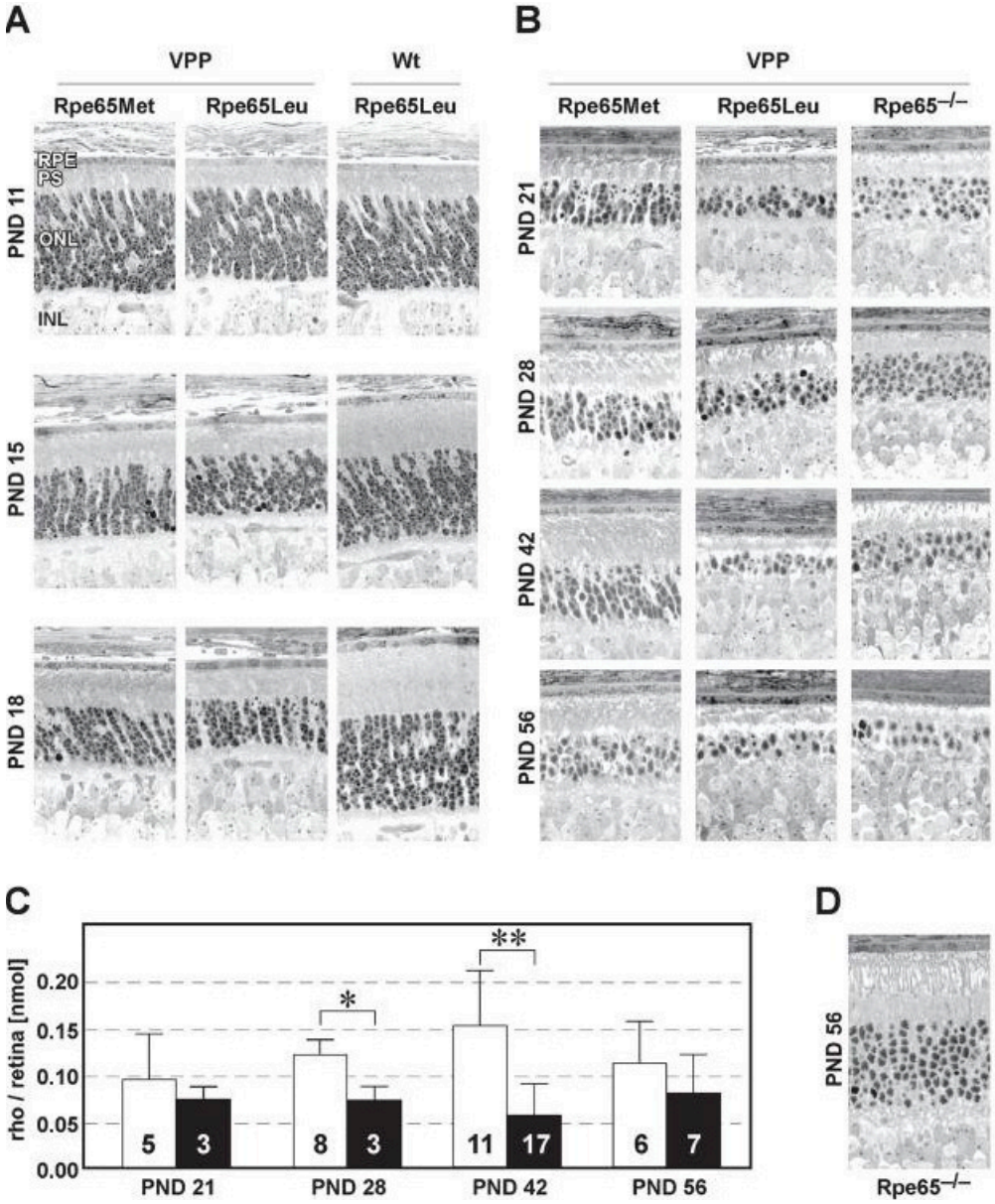

Figure 2.1. RPE65 Leu450 variant accelerates progression of retinal degeneration in a mouse model for Retinitis Pigmentosa.

Time course of *VPP*-mediated retinal degeneration was dependent on the *Rpe65* sequence. (A) Retinal morphology of *VPP;Rpe65450Met* (left column), *VPP;Rpe65450Leu* (middle column) and *wt;Rpe65450Leu* (right column) mice at PND 11 (top row), 15 (middle row) and 18 (bottom row). Shown are representative sections of the lower temporal part of at three or more independent mice. (B) Retinal morphology of *VPP;Rpe65450Met* (left column), *VPP;Rpe65450Leu* (middle column) and *Rpe65*$^{-/-}$(right column) mice at PND 21 (top row), 28 (second row), 42 (third row) and 56 (bottom row). The Rpe65450Leu variant accelerated the *VPP*- mediated retinal degeneration leading to a more pronounced thinning of the ONL and less preserved photoreceptor segments. The Rpe65 knockout only partially rescued the degeneration. Shown are representative sections of the lower temporal part of at least three independent mice. (C) Rhodopsin per retina expressed in nmol. Values are given as means + SD. Open bars, *VPP;Rpe65450Met*; solid bars, *VPP;Rpe65450Leu*. Numbers of mice are indicated. *P < 0.0046, **P < 0.0001. (D) Representative section through the lower temporal part of the retina of an *Rpe65*$^{-/-}$ mouse at PND 56. PS, photoreceptor segment; ONL, outer nuclear layer; INL, inner nuclear layer. (Samardzija et al., 2006)

2.2. THE PATHOGENIC R91W MUTATION IN RPE65 IN THE ROD-DOMINANT RETINA

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Mutations in RPE65 lead to autosomal recessive dystrophies ranging from Retinitis Pigmentosa to Lebers congenital amaurosis (Marlhens et al., 1997, Morimura et al., 1998). Missense mutations account for more than 60 % of all disease-associated mutations identified in the *RPE65* gene (128 reported to date). The pathogenic mechanisms for most of these mutations are still poorly understood. Recently, however, an *in vitro* analysis of several disease-associated mutations showed that protein misfolding and a rapid proteasomal degradation might be the pathogenic mechanism leading to blindness (Li et al., 2014, Li et al., 2015a).

Codon 91 encoding arginine (R) seems to be prone to amino acid changes as three pathogenic missense mutations have been reported in RPE65 patients at that position: R91W (Morimura et al., 1998), R91Q (Thompson et al., 2000) and R91P (Simonelli et al., 2007)). Remarkably, the R91W substitution in *RPE65* is the most frequent missense mutation found in patients in general. While R91Q and R91P patients are congenitally blind, patients carrying R91W mutation have useful cone-mediated vision in the first decade of life (El Matri et al., 2006). However, vision is completely lost in the third decade of life. Mice lacking RPE65 (*Rpe65*$^{-/-}$ and *rd12* mice) cannot synthesize 11-*cis*-retinoids, and thus present a different situation as found in patients where data suggested that some missense mutations produced versions of RPE65 with some residual enzymatic activity. To generate a mouse model that resembles the human conditions more closely, we generated *R91W* knock-in mice. These mice were used to investigate the pathophysiological

mechanisms of retinal degeneration caused by aberrant RPE65 function. The mutation caused low expression levels and reduced function of the mutant RPE65 protein in the RPE (Samardzija et al., 2008). Consistent with the predicted diminished enzymatic activity of mutant RPE65^{R91W} in patients, the *R91W* mice exhibit very low chromophore (11-*cis*-retinal) levels in the retina - accounting for less than 10% of wild-type levels. As a result of this hypomorphic RPE65 activity, rods are desensitized which leads to a severely compromised visual function. Our data indicated that the *R91W* mouse is highly suitable to study the impact of RPE65 insufficiency on rod pathophysiology. This work for the first time explained the molecular basis for differences in visual performances between RPE65 patients.

The follow up study (Samardzija et al., 2009) focused on the consequences of the R91W mutation for cone structure and function. Previous work on *Rpe65*$^{-/-}$ mice showed that cones are especially sensitive to the lack of 11-*cis*-retinal, which results in fast cone degeneration (Znoiko et al., 2005). As 11-*cis*-retinal is used as chromophore by both cones and rods, we analyzed the photoreceptor type to which the remnant visual function in *R91W* mice was attributed. We showed that limited retinal levels of 11-*cis*-retinal is especially unfavorable for the cones, which undergo a slow but progressive degeneration with a geographic prevalence. This was shown with a variety of techniques including a longitudinal *in vivo* study where we visualized GFP-expressing cone cells using a scanning laser ophthalmoscope (Samardzija et al., 2009). As a consequence of insufficient chromophore supply, most chromophore is trapped by the very abundant rod opsin leaving cone opsin 'naked'. This results in cone opsin mislocalization in the *R91W* retina. Since cone opsin is an important structural element for the cells this leads to cone degeneration and consequent loss of cone-mediated vision. This is consistent with data from RPE65 patients showing that peripheral cones, which are surrounded by rods as in the mouse retina, are much earlier affected than central cones in the rod-free foveola (Bonilha et al., 2011). The proof that rods act as chromophore 'sink' was provided by the ablation of rod opsin, which prevented cone opsin mislocalization and restored cone function in *R91W* animals (**Figure 2.2**, (Samardzija et al., 2009)). Collectively our data indicated that under conditions of limited chromophore supply rods entrap most of the available 11-*cis*-retinal leaving insufficient chromophore for an efficient cone function and cone survival. The identified competition between rods and cones for the visual chromophore suggests that it may be important to deliver a functional *RPE65* gene directly to the cone-rich macula in patients in order to avoid the strong rod chromophore trap. In addition, the fact that cones are especially sensitive to chromophore starvation adds a cautionary note to therapeutic approaches using a pharmacological inhibition of chromophore regeneration in order to reduce retinal degeneration. Such approaches may not only impair night vision of patients

but may also have negative effects on cone integrity and thus cone- mediated vision in the long-run.

In the follow up gene therapy study (Kostic et al., 2011) we showed that lentiviral vector delivery of RPE65 rescues cone photoreceptors and prevents cone degeneration in *R91W* mice. Importantly we showed that the delivered wild type copy of RPE65 rejuvenates cones that had already completely or partially lost the expression of phototransduction proteins and marker proteins of cone outer segments, respectively. The lentiviral therapy leads to long-lasting transgene expression in the RPE cells and improved retinal function. We evaluated the therapeutic window within which cones can be rescued in the *R91W* mouse and showed that this window is extended as compared to the *Rpe65*$^{-/-}$ mice (Bemelmans et al., 2006). This finding has a direct clinical relevance for the gene therapy of the patients.

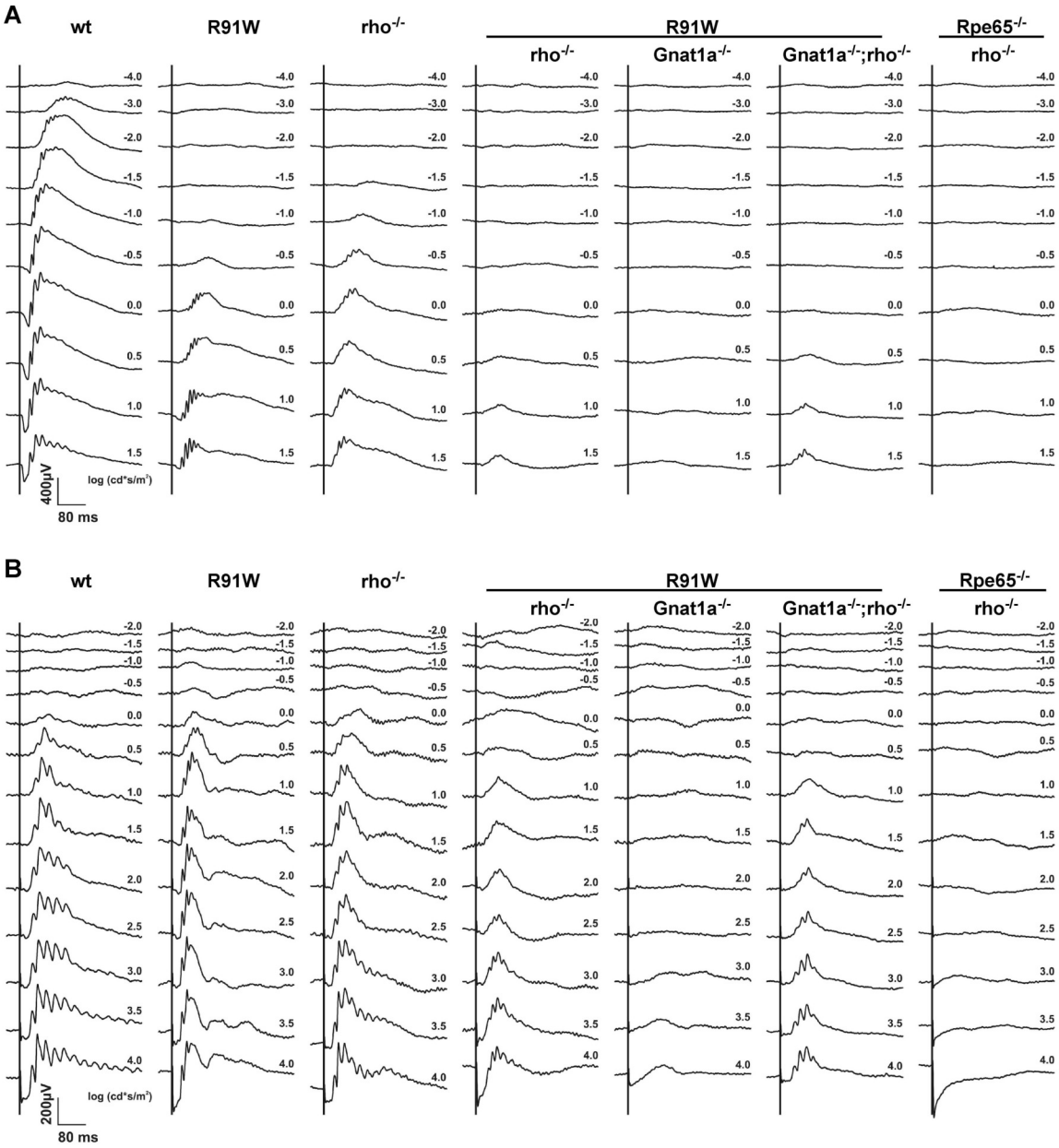

Figure 2.2. Under conditions of limited chromophore supply rods act as a sink and deprive cones form chromophore

Effect of the R91W mutation on retinal function. Evaluation of retinal function by electroretinogram (ERG) of 4-week-old *wt, R91W, rho*$^{-/-}$, *R91W;rho*$^{-/-}$, *R91W;Gnat1a*$^{-/-}$, *R91W;Gnat1a*$^{-/-}$*; rho*$^{-/-}$ and *Rpe65*$^{-/-}$*;rho*$^{-/-}$ mice. Single flash ERGs were recorded under dark-adapted (A, scotopic) and light-adapted (B, photopic) conditions. Additional deletion of rhodopsin in *R91W* and *Rpe65*$^{-/-}$ enabled the examination of pure cone function in the absence of the rod system. The vertical lines indicate the time point of the light flash. Note that an initial negative deflection in the higher intensity range (above 1.5 log cd*s/m^2) is not a retinal electrical response but a flash artifact. (Samardzija et al., 2009)

2.3. THE R91W MUTATION IN RPE65 AND CONES

7. Samardzija, M, C Caprara, SR Heynen, S Willcox DeParis, I Meneau, G Traber, C Agca, J von Lintig, and C Grimm. A mouse model for studying cone photoreceptor pathologies. ***Invest Ophthalmol Vis Sci.*** **2014; 55:5304-5313.**

8. Geiger, P, M Barben, C Grimm, and M Samardzija. Blue light-induced retinal lesions, intraretinal vascular leakage and edema formation in the all-cone mouse retina. ***Cell Death Dis.*** **accepted;**

Considering that the rod-cone ratio in the human macula is much in favor of cones, with the central fovea lacking rods altogether, the results obtained in the rod-dominated retinas of *R91W* mice phenocopy the situation in the peripheral but not central retina of patients. To generate a model that is suited to study the impact of the R91W mutation on cones in the central macula, we combined the *R91W* with the *Nrl*$^{-/-}$ mouse (Mears et al., 2001). The *Nrl*$^{-/-}$ mice lack the neural retina leucine zipper (NRL) transcription factor, necessary to direct photoreceptor progenitor cells towards the rod cell fate. *Nrl*$^{-/-}$ retinas are overpopulated with an excess of cone photoreceptors and lack rods completely. Therefore, the retina of *R91W;Nrl*$^{-/-}$ double mutant mice mimics the situation in the central, cone-rich, rod-free retina of human patients suffering from a hypomorphic RPE65 function. Importantly, cones of *R91W;Nrl*$^{-/-}$ mice are functional and show a strong photopic activity. We showed that despite the severely reduced amount of chromophore in *R91W;Nrl*$^{-/-}$ mice, the cones survive much longer in such a cone-only environment than in the rod-dominant retina of *R91W* mice (Samardzija et al., 2014). This is consistent with the prolonged survival of foveal cones in RPE65 patients and suggests that the *R91W* and the *R91W;Nrl*$^{-/-}$ mouse lines are genuine models to study human pathologies caused by the *Rpe65*^{R91W} mutation in the peripheral and the central retina, respectively.

To investigate the molecular fingerprint of cone degenerations we further generated mutation-mediated (inherited) cone degeneration models by crossing *R91W;Nrl*$^{-/-}$ mice with *Cpfl1* (Chang et al., 2002) and *rd10* (Chang et al., 2002) mice resulting in triple and quadruple mutant mice, respectively. While triple mutant *R91W;Nrl*$^{-/-}$*;Cpfl1* mice still had a good retinal function, retinas of quadruple mutant *R91W;Nrl*$^{-/-}$*;Cpfl1;rd10* mice were devoid of function. All mice exhibited a degeneration of the cells in the outer nucler layer, albeit with different kinetics and severities. The quadruple mutant mice showed the most severe degeneration, the triple mutant mouse an intermediate degeneration and the original

R91W;Nrl$^{-/-}$ mouse had the slowest degeneration kinetics. The comparative study of degenerations in these models is currently under thorough investigation in the laboratory aiming at the identification of the molecular pathways responsible for cone cell death.

In the final study (Geiger et al., accepted) the consequences of exposure to toxic levels of blue light for the all-cone retina were analyzed. It is important to note that *R91W;Nrl*$^{-/-}$ mice are resistant to standard light damage protocols that use high levels of white fluorescent light. As expected from the studies described above, reduced levels of RPE65^{R91W} protein together with extremely low chromophore yield (Samardzija et al., 2008) renders *R91W;Nrl*$^{-/-}$ resistant to white light. In contrast to white light, blue light strongly damaged *R91W;Nrl*$^{-/-}$ retinas. In general, light of shorter wavelengths is more harmful to the retina than long wavelength light. It has been clearly demonstrated in rodents, rabbits and monkey that the damage threshold correlates with the wavelength of light used for exposure: the shorter the wavelength, the lower the threshold (van Norren and Gorgels, 2011). Furthermore, *R91W;Nrl*$^{-/-}$ retinas are overpopulated with short wavelength cones which absorb light maximally in the UV range at 360 nm, which is close to the wavelength of the damaging blue light used in the study (410 nm). In addition, blue light triggers photochemical regeneration of bleachable rhodopsin on site – in the photoreceptor outer segments – without the involvement of metabolic regeneration via the visual cycle and RPE (Williams, 1964). This process – termed photoreversal of bleaching – leads to a dramatic increase in the photon catch capacity by the visual pigment in blue light conditions (Grimm et al., 2000). In the wild-type rod-dominant mouse retinas blue light induced predominantly the loss of rod photoreceptor cells and disruption of the outer retinal blood barrier that is composed of RPE cells in retina. We showed that toxic levels of blue light in *R91W;Nrl*$^{-/-}$ mice not only induces cone photoreceptor death but also affects other cells especially of the inner retina and causes a strong microglial response as well as neuroinflammation. We found strongly increased levels of *Tnf* and *Il1b* in the all-cone retinas of *R91W;Nrl*$^{-/-}$ as compared to *wt*. It is important to note that microglial activation impairs blood brain barrier function by the release of various proinflammatory factors, including TNF and IL1B, shown to enhance retinal vascular permeability (Abcouwer et al., 2013) but also hyperpermeability associated with neurodegenerative disorders such as Alzheimers disease and multiple sclerosis (reviewed in (da Fonseca et al., 2014). Upregulation of *Tnf* and *Il1b* expression in response to blue light exposure was paralleled by vessel leakage leading to retinal hemorrhages, suggesting that the inner retinal blood barrier was affected in the all-cone retina. We also provide evidence for a strong edema formation after the light insult in *R91W;Nrl*$^{-/-}$ mice (**Figure 2.3**; (Geiger et al., accepted)). Diabetic macular edema is characterized by low-grade inflammatory reaction, a blood-retinal barrier breakdown and retinal capillary leakage (Klaassen et al., 2013). Thus blue light damage in *R91W;Nrl*$^{-/-}$ mice

can be used to test potential therapeutic agents for the treatment of edema-based complications in macular degenerations.

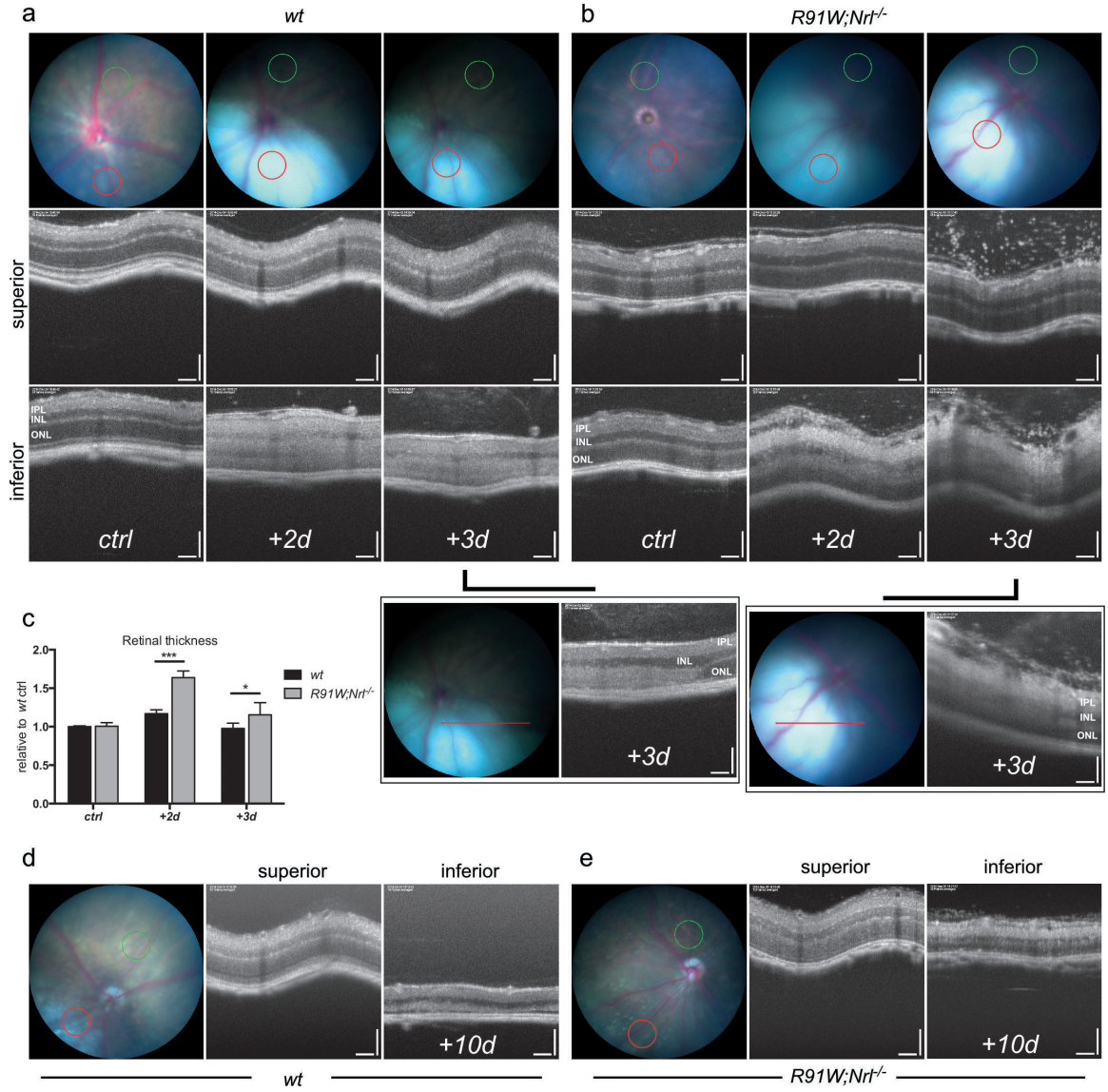

Figure 2.3. *R91W;Nrl*$^{-/-}$ as a model for macular edema

Fundus (color) and corresponding OCT (black and white) images of *wt* (a, d) and *R91W;Nrl*$^{-/-}$ (b, e) mice taken up to 10 days following blue light damage (BLD). The positions of the OCT scans are shown in fundi as colored circles/lines (green, superior; red, inferior). At 2 and 3 days after BLD the hot spot regions appeared as a pale bluish spot, much lighter than the rest of the fundus (a, b). OCT revealed that INL and ONL in the damaged (inferior) but not the control area (superior) became hyperreflective in *wt*; while hyperreflectivity was very pronounced in the IPL but absent in the ONL in *R91W;Nrl*$^{-/-}$ mice. Boxed panels in (a) and (b) show linear scans of the transition zones analyzed 3 days following BLD. Increased retinal thickness was especially prominent in *R91W;Nrl*$^{-/-}$ mice. Quantification of retinal thickness in *R91W;Nrl*$^{-/-}$ and *wt* eyes that were unexposed (ctrl) or exposed to blue light, as indicated (c). Values are expressed relative to the mean value of unexposed *wt* mice that was set to 1. N = 4 (*wt*) or 5 (*R91W;Nrl*$^{-/-}$); *P<0.05; ***P<0.001). At 10 days after BLD the hot spot regions can be recognized by whitish material appearing in the fundus and by a thinned retina in OCT (d, e). Note: The 2 d and 3 d time points in panels (a) and (b) show data from the same mouse followed for two consecutive days. Scale bars 100 μm. (Geiger et al., accepted)

CONCLUSION

Currently, no therapies are available to prevent photoreceptor death. This is partially due to a limited knowledge of the mechanisms by which photoreceptor cells die. Even less is known about specific mechanisms of cone degeneration in age related-macular degeneration. Our research, which started with experiments aiming to understand the basic role of RPE65 in the visual cycle, led us to the development of *R91W* and *R91W;Nrl*$^{-/-}$ mouse models. While the *R91W* single mutant mouse served as a base for understanding pathogenic mechanisms underlying RPE65 insufficiency, the *R91W;Nrl*$^{-/-}$ double mutant mouse allowed the investigation of the consequences of disease causing cone-specific mutations in an organized all-cone environment. The *R91W;Nrl*$^{-/-}$ mice are especially suited for neuroprotective studies, gene therapy approaches and for cone cell transplantation experiments to rescue cone vision. Several research groups already acquired the *R91W* and *R91W;Nrl*$^{-/-}$ mouse lines for projects such as in-depth analysis of RPE65^{R91W} biochemistry (M. Jin, LSU Health Sciences Center School of Medicine, New Orleans, US); MRI imaging of cone physiology (B. Berkowitz, Wayne State University, Detroit, US); cone transplantation approaches (R. Ali, University College London, UK); electrophysiology of synaptic transmission in cone photoreceptors (S. Baker, University of Iowa; US). Thus, the mice will significantly accelerate cone related research and the collection of data needed to develop successful therapeutic strategies to treat cone related retinal degenerations in human patients.

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